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FOREST INSECT INVESTIGATIONS

ARTIFICIAL PROPAGATION OF TWO NATIVE PREDATORS
OF THE MOUNTAIN PINE BEETLE IN SUGAR PINE

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Forest Insect Laboratory
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TABLE OF CONTENTS

INTRODUCTION	1
DEVELOPMENT OF TECHNIC	2
<u>Source of Adults</u>	2
<u>Selection of Mated Pairs</u>	2
<u>Oviposition Setup</u>	3
<u>Collection and Incubation of Eggs</u>	3
<u>Rearing Setup for Larvae</u>	4
<u>Feeding the Larvae</u>	4
<u>Source of Host Food</u>	4
<u>Facilities in Conducting Experiments</u>	5
ADAPTABILITY OF PREDATORS TO ARTIFICIAL PROPAGATION	5
<u>Longevity of Adults</u>	5
<u>Fecundity of Females</u>	6
<u>Seasonal Production of Eggs</u>	7
<u>Viability of Eggs</u>	8
<u>Development of Larvae</u>	8
<u>Mortality of Larvae</u>	9
<u>Possibility of Development on Foreign Hosts</u>	10
PRACTICAL LIMITATIONS IN ARTIFICIAL PROPAGATION	11
<u>Time and Cost</u>	11
<u>Host Food Supply</u>	13
<u>Cannibalism</u>	13
<u>Life Cycle in Relation to Host</u>	14
SUGGESTIONS FOR FURTHER STUDY	14
SUMMARY	15
CHARTS AND PHOTOGRAPHS	

ARTIFICIAL PROPAGATION OF TWO NATIVE PREDATORS OF THE MOUNTAIN PINE BEETLE IN SUGAR PINE.

INTRODUCTION

Recent studies to develop methods for the biological control of the mountain pine beetle in sugar pine through the use of native predators have been directed along two lines of attack; first, an evaluation of the effectiveness of the two most common species of predators and of the possibilities for protecting these insects in the woods through modification of existing control methods; second, to determine whether there is any possibility of rearing these insects under laboratory control in sufficient numbers to use them as a controlling factor when liberated. Progress in the first phase of this study has been presented in a recent report. The present report deals with the progress of laboratory rearing experiments.

Inasmuch as the two species of predators included in this work are native and are everywhere established throughout the range of the mountain pine beetle, little can be expected from artificial or insectary rearing unless methods are found for producing these predators more efficiently than is the case in nature. The lines of attack that have been considered in experimentation to date are:

1. Elimination of competition for food and prevention of cannibalism such as occurs to a considerable extent in infested trees.
2. Substitution of a more available food supply such as fly larvae in the place of Pandroctonus broods during the period of larval feeding.
3. Possibility of maintaining large numbers of predators under insectary conditions during periods of low barkbeetle activity so that they can be used for liberation at the first sign of a local barkbeetle outbreak.

Before much progress can be made in the development and practice of biological control through laboratory rearing methods, it is essential first of all to understand how the development of the predator coincides with the life history of the mountain pine beetle; what factors characteristic of a given predator will work to a greater advantage over another; and what are the practical limitations of artificial propagation. In the development of a laboratory program for this study, careful consideration has been given to these qualifications.

The red bellied clerid, Enoclerus apageus, and the green trogositid, Tannochila virescens, were selected for this study only after

field data and controlled rearing experiments had fairly definitely established them as the two most promising natural enemies. The work carried on thus far, in addition to determining the factors as mentioned, has been comparative in nature to establish one or both predators for use in biological control.

The initial work on artificial propagation was carried on at Berkeley, California where cold storage and constant temperature rearing facilities at the University of California were highly favorable to this type of investigation. This was supplemented by further experimentation under natural field conditions at the Miami Laboratory established on the Sierra National Forest. (Photo 1) Studies were begun in October, 1937, and are still in progress.

The experiments thus far have uncovered some of the limitations of artificial propagation. Techniques and methods have been developed, the successes of which have given greater encouragement to continue. The progress and accomplishments on artificial propagation completed to date are herein presented.

DEVELOPMENT OF TECHNIC

Source of Adults

Predator adults are normally attracted to the main boles of sugar pines freshly attacked by the mountain pine beetle, where they prey upon the attacking beetles and lay their eggs which hatch and feed upon the pine beetle broods. Hence, it was here that the search for laboratory specimens was carried on. The best collecting was obtained from windfalls which served as trap trees, where often great numbers of T. virescens and E. ophegus adults were found. E. ophegus adults were readily found during May and June, but rarely after the first of July, while T. virescens adults were easily obtainable at any time throughout the summer.

Additional E. ophegus adults were secured by first collecting the mature larvae after they had migrated. These were placed in quart jars (50 larvae in each jar) partly filled with dry sawdust. Pupal cells were readily constructed followed by the normal aestivation period. Mature larvae thus collected during July gave rise to adults as needed for experimental use during the last week in August and throughout September.

Selection of Mated Pairs.

Early in the development of artificial rearing technic, it was found that the treatment of single mated pairs as a unit was much more satisfactory than placing numbers of males and females in the same container where fighting often resulted in loss of individuals. Owing to the

apparent lack of any external character to be used in segregating sexes of either species of predator, the common procedure in selecting mated pairs was to place a number of adults in a petri dish. (Photo 3) At room temperature these would mate readily and the pairs thus inclined were separated into individual petri dishes.

Oviposition Setup

The mated pairs were then set up to induce oviposition by placing a small flat block of green sugar pine or ponderosa pine bark with the phloem and a thin strip of sapwood attached within the dish. (Photo 4) A few mountain pine beetle adults (usually 6) were added to the setup for predator food and to attack the blocks, and thus simulate natural conditions favoring predator oviposition. Following each setup the predators were left for a period of one week, when each block was removed and checked over for eggs. Each week new blocks were set up with more D. monticolae adults, and this procedure was continued until the egg-laying period was completed.

As the above procedure is laborious and time consuming, other methods were tried to induce oviposition. By far the best method developed was the elimination of pine blocks and in place of them, the use of small crepe paper rolls tightly wound. (Photo 5) These are placed in a small jar containing a mated predator pair and a few D. monticolae adults. Eggs were deposited normally between the layers of paper made by the roll, and were readily collected. The use of different-colored crepe paper made no difference in the tendency to oviposit, hence white paper was chosen as best since the various dyes in the colored paper apparently had a damaging effect on the eggs, especially in a moist chamber.

Collection and Incubation of Eggs

Eggs were removed from the pine block by the laborious procedure of flicking away all the bark scales, and examining every crevice. Often as many as 30 eggs in a group were uncovered. (Photo 6) Utmost care in uncovering the eggs was found to be essential to prevent crushing. With the use of crepe paper rolls, the eggs are simply collected by unrolling the paper and clipping off the egg groups.

The eggs collected each week were transferred to a petri dish on a piece of blotting paper and left to incubate. A camel's hair brush was found to be essential in the transfer of individual eggs. At a constant temperature of 75° F. they hatched in from 5 to 7 days; at outside summer temperatures the usual time required was 8 to 10 days.

* Detailed studies made recently on both species have resulted in the location of definite external sexual characters readily distinguishable with a hand lens. These characters are described in a laboratory memorandum by L. H. Carpenter. This discovery will aid materially in setting up subsequent experiments.

Rearing Setup for Larvae (Photos 7 and 8)

Because of the cannibalistic habits of both species of predators it was not possible in the development of numerous methods of rearing to propagate more than one individual in a container and expect them to reach maturity. The method finally adopted and applied with success was developed through the use of large numbers of individual containers. The best containers found were pharmaceutical shoulder-lip vials of one dram capacity. These were most adaptable because the shoulder prevents the larvae from escaping. Other qualities such as the flat bottom, and a size adaptable to storage in large numbers were in addition found to be advantageous.

As soon as the larvae hatched from the eggs in the petri dish incubation chamber they were transferred by means of a camel's hair brush to the vials, each containing a small amount of fine sawdust*. The open vials were as a rule set in containers of 100 each and left this way throughout the feeding period.

Feeding the Larvae

In common practice the larvae were fed every other day from the beginning of each setup until maturity. Mature scolytid larvae were fed even to the smallest, first-stage predator larvae, since these were readily eaten and caused the predators to grow faster. In feeding the larvae, the simple procedure of dropping one host larva in each vial was followed. The predator larva always found its prey.

Source of Host Food

The greatest amount of host food was obtained through rearing mountain pine beetle larvae in green logs by enclosing them in cages large enough to accommodate 20 or more 12-inch logs 4 feet long. Attacks were induced by liberating D. monticolae adults, usually at a rate of 15 adults to the square foot of bark area. Broods of mature larvae varying in number from 150 to 300 per square foot were usually ready to be used as predator food in from 4 to 6 weeks after inducing attacks.

The D. monticolae adults to be used in inducing attacks and for adult predator food were obtained by rearing them out of sugar pine logs or slabs which contained the mountain pine beetle broods. These were secured through felling infested trees attacked in the field early in the season. Enough adults were secured in this way from overwintering broods to carry on experiments during the remainder of the field season by storing surplus numbers under refrigeration and using them as needed.

*The rearing environment of dry sawdust was found much more satisfactory than a moist environment, since the latter always invited fungus contamination which resulted in high mortality. Mortality in the dry environment was never more than 7 percent.

Other host foods to be used in lieu of or supplemental to the native scolytid host were obtained by rearing them in the laboratory on culture media suitable for normal development. Wheat bran provided an excellent rearing medium for grain beetles and moths. Potatoes were used in culturing the potato tuber moth; bees wax in culturing the greater wax moth. All such rearings were carried on at Berkeley at 75° F. Battery jars were found to be suitable containers for this purpose. Flesh fly larvae, *Lucilia sericata*, most promising of any of the hosts used, were supplied by the entomology department of the University of California.

Facilities in Conducting Experiments

1. Refrigeration. Cold storage facilities at the University of California materially benefited the experimental work at Berkeley. Surplus predators and a large supply of mountain pine beetle adults were stored at 36° F. and used as needed in setting up experiments. At the Miami field laboratory, refrigeration was maintained with an Electrolux gas refrigerator (Photo 2) which served the same purpose.

2. Laboratory. At Berkeley all the rearing work was carried on at a constant temperature of 75° F. maintained by a specially constructed room in Giannini Hall, heated by an electric light. In the field, the rearing work was carried on inside the Miami Laboratory, but no provision was made for heating the building. Hence, at night the temperature inside was only slightly higher than the outside temperature. The heat given off by the Electrolux refrigerator tended to keep the temperature up somewhat.

3. Cooperation. The entomology department at the University of California supplied all the fly larvae needed in testing them as host food for predators. A weekly supply of fly larvae was provided for the full term of experiments at Berkeley, which have been carried on for somewhat more than one year. Additional foreign host material from which to start cultures was also provided by the University. These were the mediterranean flour moth, Indian meal moth, greater wax moth, potato tuber moth, and the meal worm.

4. Assistance. The work at Berkeley was assisted by S. C. Dorman, Elwyn Dorman, Neil Stanger and Etsy Schneider. Mrs. Schneider, engaged as Minor Scientific Aide, carried on all the laboratory tests at Berkeley during the summer and fall of 1938, and is still employed on this work. Field assistance at Miami was provided by Messrs. L. B. Carpelan and L. R. Gillogly.

ADAPTABILITY OF PREDATORS TO ARTIFICIAL PROPAGATION

Longevity of Adults

In an artificial environment, as well as under normal field conditions, the life period of an adult predator will vary according to

the environmental factors of temperature, humidity and food supply. But even under the most favorable conditions, individual differences, possibly inherent in the species, cause a great range in life period. This fact has been demonstrated in all experiments carried on thus far wherein the environment was kept as constant as possible.

The life period of the adult is divided into two distinct phases: (1) the pre-adult period, following emergence from the pupal stage, in which the adults are not sexually mature*; and (2) the oviposition period which is 3 to 4 times longer for T. virescens than for E. aphegus.

The second phase of adult life for each species is best illustrated by laboratory oviposition tests of each species. Six females out of a total of 10 mated pairs of E. virescens, set up at a constant temperature of 75° F. on March 4, 1938, continued to oviposit on November 20, 1938, over 8 months later. The minimum oviposition period for a member of this same series was 3½ months. On the other hand, the maximum length of active life as a mature, egg-laying E. aphegus adult was 2½ months, and the productive egg-laying period was actually shorter than this, since during the last few weeks the number of eggs laid fell off markedly.

Under the confined conditions provided by the artificial setup for oviposition, the E. aphegus female as a rule kills and eats her mate after 6 weeks. Whether this is a normal habit has not been determined. Oviposition is continued for, at most, a month after this only when the female is remated with another male.

Fecundity of Females

From records based on 59 mated pairs of T. virescens, producing 6,334 eggs over a period of slightly more than a year, the maximum number of eggs laid by one female was 581 over a period of 8 months; the minimum number laid by one female was 125 eggs in a period of 3½ months. One series, laying eggs over a period of 4 months produced a maximum of 232 eggs (or 14.6 eggs per week) and a minimum of 38 eggs (or 2.5 eggs per week).

From records based on 31 mated pairs of E. aphegus, producing a total of 1,573 eggs over a period of 7 months, the maximum number of eggs laid by one female was 93 over a period of 9 weeks (or 10.33 per week). The minimum was 5 eggs produced in 6 weeks (or less than 1 per week).

In comparing the average egg production per week for the two species, T. virescens females were found to have laid an average of 5.7 eggs per week while the E. aphegus females laid an average of 6.2 eggs

* In both species this period varies from 3 to 5 weeks during which time they apparently must feed on scolytid adults to attain maturity.

per week. However, the average number of eggs per female is 107 for 50 T. virescens whose egg laying period varied from 3½ to 8 months, while the average number of eggs for the 31 E. aphegus was 51, during an egg laying period of 1½ to 2½ months. A summarization of this data follows:

Table 1. Comparative Egg Production of Predators.

Species	Maximum no. eggs per female	Min. no. eggs per female	Ovipos- ition period	Av. no. eggs per week	Av. no. eggs per female
<u>T. virescens</u> :	561	38	3½-8 mos.	5.7	107
<u>E. aphegus</u> :	93	5	1½-2½ "	6.2	51

Seasonal Production of Eggs

Under laboratory rearing conditions the number of eggs laid by a given group of mated predator pairs for the total production period of the group has shown in all cases a gradual increase to a peak, followed by a gradual to sudden decline and final cessation. The best example of this habit is shown by the line graph (Figure 1) of the oviposition experiments carried on at Miami, comparing T. virescens and E. aphegus. Both of these predator groups were set up two weeks previous to the date of the first egg collection on June 30. In the case of each predator the production builds up to a certain point, then drops off. This point was reached by E. aphegus on July 28 and by T. virescens on August 19. Egg production ended for E. aphegus on August 25 and for T. virescens on September 30. This data is summarized in Table 2 which follows.

Table 2. Seasonal Weekly Egg Production of T. virescens and E. aphegus.

Species	Number of eggs laid each date											
	June			July			August			September		
	30	7	14	21	28	4	11	19	25	1	8	15
<u>T.v.c.</u>												
Ser. A:	59	100	37	97	103	170	284	235	45	47	53	52
Ser. B:	24	62	39	5	33	13	61	60	21	86	42	16
Ser. C:	45	62	44	71	48	102	145	81	53	31	60	15
Total	128	224	120	173	184	285	490	376	129	164	155	83
<u>E. apheg.</u>												
Ser. A:	47	92	122	129	67	110	37	18	1			
Ser. B:	23	49	87	42	77	44	27	14	13			
Total	70	141	209	171	164	154	64	32	14			

Viability of Eggs

Tests on both T. virescens and E. sphagnum for egg viability are too few and of such a character that they cannot be considered conclusive. Three tests involving a total of 125 Tennochila eggs, of which 86 hatched, indicate a mortality of 31 percent (viability 69 percent). Three tests on a total of 28 E. sphagnum eggs gave a mortality of 46 percent (viability 54 percent).

Such factors as desiccation and possible damage in removal from the bark crevices may have caused this high mortality. But the tests indicate that under the conditions of the experiments, a viability between 50 and 70 percent can be expected. A tabulation of the data from the tests conducted on both species of predators is presented below.

Table 3. Egg Viability Tests of T. virescens and E. sphagnum

Species	Test #	Total eggs tested	No. hatched	Fertility %	Av. fertility
<u>T. v. g.</u>	1	31	23	74	69
"	2	25	19	76	
"	3	69	44	64	
<u>E. sphag.</u>	1	10	7	70	54
"	2	14	6	43	
"	3	4	2	50	

Development of Larvae

The time spent in the larval stage is of importance because the length of feeding period is the most time consuming, and hence the most costly phase of artificial propagation. At a constant temperature of 75°F. the shortest time observed to be required by T. virescens to complete its life cycle was 10 to 12 weeks by five members of a series of 100 larvae set up March 14, 1938, and 6 members of a series of 58 larvae set up on May 25, 1938. All the larvae remaining in the former series pupated between November 22 and December 19, 1938 and the average elapsed time from the beginning of the setup was 9 months. Figure 2 represents graphically the development of this series which is typical of the development under laboratory conditions at a constant temperature of 75° F. All the larvae remaining in the latter series showed no sign of pupation 6 months after they had been set up, and these were placed in cold storage to hibernate, owing to a shortage of host food.

The larvae remaining in both series, following the early pupations, continued to feed and moult every 5 to 10 days. This behavior is

similar to that of nearly all the other T. virescens larvae observed. In all cases of rapid development in the larval stage, 5 moults were required prior to the pupal stage. This is in definite contrast to the number of moults taking place by larvae living for long periods before pupation. While exact data on the maximum number is lacking, it is estimated that in many cases, as many as 10 to 15 moults occurred in certain individuals before pupation. The possibility of certain factors caused by artificial propagation coming in and altering the normal habits of larvae have yet to be investigated. However, it is doubtful that this is different from the normal habit.

The active larval period of E. sphegus in all rearing tests varied between 4 and 6 weeks, with two moults required before pupation. The fully fed second stage larvae always constructed their pupal cells and remained quiet for periods varying between 3 and 4 weeks before pupating. Under the artificial rearing conditions the total rearing period from egg to adult was never found to be longer than 12 weeks at 75° F. and under the summer rearing temperatures at the Miami laboratory. (See chart of maximum and minimum temperatures, Figure 3.)

The results of all laboratory rearing experiments with both species of predators to date have shown the average and minimum periods required for the development of each stage as presented below.

Table 4. Comparative Development of T. virescens and E. sphegus

Species :	Incubation :	Larval :	Prepupal :	Pupal period:	Total time
	period days:	period days:	period days:	days :	days
	Av. : Min. :	Av. : Min. :	Av. : Min. :	Av. : Min. :	Av. : Min. :
<u>T.v.c.</u> :	7 : 5 :	240 : 50 :	10 : 6 :	12 : 10 :	256 : 58 :
<u>E.spheg.</u> :	8 : 6 :	46 : 23 :	30 : 20 :	31 : 8 :	118 : 51 :

Mortality of Larvae

The survival of predator larvae under the artificial conditions developed thus far is dependent mainly on the kind and quality of host food. When fed on their natural host (scolytid larvae) the mortality for T. virescens was between 5 and 10 percent. For example, the mortality among 1083 newly hatched larvae which were fed on B. monticolae larvae for a period of 3 months was slightly less than 7 percent. Another group of 58 which were fed on B. brevicornis larvae showed up a mortality of 9 percent at maturity. When fed on fly larvae (Lucilia sericata) the mortality of 100 T. virescens larvae in one test was 96 percent at maturity. In two other tests with T. virescens, using L. sericata as host food, the mortality at maturity was 80 and 91 percent respectively. A summary of

tests conducted to show the relation of host food to mortality of T. virescens is presented in Table 5.

Table 5. Survival of T. virescens in Relation to Host

Host food	Test series	Period days	No. larvae set up	Survivors	% mortality
<u>L. sericata</u>	B-B	440	106	9	91
"	B-C	410	146	28	80
"	B-E	175	100	4	96
"	B-5	168	100	15	85
<u>D. brevicornis</u>	B-6	168	58	53	9
<u>D. monticolae</u>	M-a to j	122	1083	1010	7

Larval mortality of T. virescens was found to be highest among the young first and second stages, during the first 2 weeks of their existence. Hence the kind of host food given during this period is a critical factor in artificial propagation. The basis for this assertion was determined by one test (Series B-E) of 100 larvae which were fed for the first 2 weeks on D. brevicornis larvae, followed by fly larvae for the remaining period of the experiment. In this test, set up on July 21, 1938, seventy of the larvae remained alive and apparently normal at the end of 194 days. One of these larvae turned to the prepupal stage after 201 days.

The survival of E. aphegus larvae is noticeably less than T. virescens even on natural host food. For example, the mortality for one group of 537 larvae set up on D. monticolae amounted to 12.5 percent. The mortality of another group of 80 larvae which were fed on D. brevicornis amounted to 11.2 percent. On all hosts other than scolytid larvae the mortality of E. aphegus was 100 percent.

Feasibility of Development on Foreign Hosts

Experiments have shown definitely that E. aphegus is not adaptable to development on any except its natural host food. On the other hand, T. virescens has indicated adaptability to a number of insect hosts totally unrelated to the natural food. Therefore, any plan to develop a satisfactory and practical method of artificial propagation on foreign hosts is applicable only to one species.

The use of foreign insect hosts is much more desirable than the costly procedure of rearing predator larvae on native host food, provided the host selected can be reared simply and in large quantity and the survival of predators is high. The larvae of the fly, Lucilia sericata, have proven to be the most satisfactory of all host material yet found.

They can be obtained in great numbers in a small amount of substratum so that little time is lost in collecting them; and are obtainable at any season, at low cost and in a very short space of time.

The only difficulty seen in the use of flesh fly larvae as food is the high mortality of young T. virescens larvae. However, if these predators are reared for the first 2 weeks of their life on native host food and later placed on a diet of fly larvae, the mortality factor is greatly lessened. Such procedure would be a vast improvement over present technique from the standpoint of practicality.

The use of other hosts in artificial rearing experiments have so far proven to be unsatisfactory. Those tried out in order of success were the potato tuber moth, greater wax moth, Mediterranean flour moth, Indian meal moth, granary weevil, confused flour beetle and the saw-tooth grain beetle. The first three of these showed some promise, but the main difficulty was apparent in providing a suitable substratum for predator and host, or one from which the larvae could be easily obtained. The second disadvantage was apparent in maintaining sufficient populations at all times during the development period of the predator. In both respects, flesh fly larvae have a distinct advantage.

PRACTICAL LIMITATIONS IN ARTIFICIAL PROPAGATION

Time and Cost

The success of any method of artificial propagation of predators depends largely on the cost of rearing from eggs to adults. The method to be adopted finally will be the one by which the greatest number are reared for the least amount of money. The experimental work carried on thus far has been developed with these basic fundamentals in mind. The technique which have been adopted are, at best, costly, but their success from the rearing standpoint is excellent, and with several refinements already developed, the cost can be reduced considerably.

All the information on rearing time is based on the propagation of 1,000 T. virescens and 500 E. sphegus larvae, reared at the Miami laboratory using D. monticolae larvae exclusively as food. Technique have been improved since these rearings were made and hence the time estimates given herein are somewhat high. For example, the method employed for oviposition, using blocks of bark, has since been supplanted by crepe paper rolls. This single change is not only a definite time saver, but is also a vast improvement in technic.

The amount of time estimated to carry out each step of the rearing of 1,000 T. virescens and 1,000 E. sphegus, based on the Miami records, is presented in table 6. For purposes of comparison, the time required to handle both the long and short lived T. virescens larvae is included in the table.

Table 6. Time Requirements for Artificial Propagation,
Using Scolytid Larvae as Host Food, for
Each Predator

Operations involved.	1000 T.v.c	1000 T.v.c	1000
	short term	long term	E. aphegus
	0 weeks	15 weeks	6 weeks
	man hours	man hours	man hours
Securing predator adults for oviposition	32	32	32
Setting 50 mated pairs (oviposition)	2	2	2
Preparing pine blocks (oviposition)	5	5	5
Adding D.n. adults to blocks (oviposition)	6	6	6
Collecting predator eggs	30	30	30
Artificial rearing setup for larvae	10	10	10
Log set up to rear host	18	30	12
Rearing adult scolytids (D.n.)	16	32	16
Collecting adult scolytids	9	15	6
Collecting scolytid larvae for food	64	100	40
Feeding predators (every other day)	32	50	20
Observations and mortality checks	9	15	6
Total man hours	233	327	185
Total Man Days	29	41	23

It is to be noted that the most time consuming phases of artificial propagation are taken up by the preparation and collection of host food. Therefore, the development and use of a host food which can be more easily reared in quantity and one which will take less time to handle is much more desirable. The use of flesh fly larvae after the first 2 weeks of feeding would partly obviate such a handicap, but there would still be the necessity of keeping enough scolytid larvae on hand to feed the younger larvae. The use of flesh fly larvae (applicable only to the rearing of *T. virescens*) is calculated to reduce the total time of rearing by approximately 25 percent.

The improvement of other phases of artificial propagation, making use of recent improvements in technic, will reduce the total time by an additional 10 percent. For example, the time involved in selecting mating pairs can be reduced by segregating sexes, which has heretofore been impossible; and by use of crepe paper rolls for oviposition. The time spent in setting up for oviposition and collecting the eggs should be greatly reduced by application of the new method. The time taken up in the field collecting adult predators can be eliminated after the original setup, since all adults used in continued propagations will be taken from laboratory reared specimens.

Even with the application of all improvements of rearing technic, it is possible that still further savings in time can be made through rearing in large quantity. For instance, 10,000 larvae can be taken care of in less time per 1,000 than a single setup of 1,000 larvae, by the employment of more efficient methods of handling adaptable to large scale rearing.

Host Food Supply

The use of flesh fly larvae as a supplemental host food to scolytid larvae appears to offer some promise; but there is still great need for checking the results of its use through the complete development of T. virescens larvae. The provision for a suitable rearing setup for flies, considering the sanitation angle will require some thought. However, they are easily reared under almost any setup when provided with a suitable substratum, such as fish heads.

If no host other than scolytids is found to be feasible for rearing purposes, then artificial propagation of native predators for biological control is practically out of the question. For, in order to rear 1,000 T. virescens larvae to maturity, considering the average period for development of 200 or more days, and that each larva is fed every other day, 100,000 scolytid larvae would be required. This figure, translated in terms of a logging operation would involve the use of somewhat more than 200 logs, having an average bark area of $\frac{1}{2}$ square feet. In terms of volume this would be approximately 5,000 board feet. This amount of lumber is produced by a tree 46 inches D.B.H. having $\frac{1}{2}$ logs.

Cannibalism

The cannibalistic habits of both species of predators is a factor of greatest importance to mass artificial rearing. In the limited space which would be required to rear large numbers of larvae, cannibalism, even in an abundant supply of host food, is very high. This habit was best illustrated in a laboratory experiment at Berkeley, wherein out of a total number of 140 new T. virescens larvae set up and distributed evenly among 16 medium sized containers filled with corrugated paper cells which were kept full of scolytid larvae, only 16 reached maturity, pupated and turned to adults. In other words only one larva in each container reached maturity. This same result was obtained from 10 containers of 100 E. sphagnum larvae, also kept supplied with native host food.

The use of individual containers to eliminate cannibalism is the only means yet developed to insure success in artificial propagation. But even with greater refinements developed, making this means of rearing within the practical limitations of cost, there is the ever present factor of cannibalism taking place in nature which cannot be controlled. Thus if the total predator population is increased many times through laboratory practice, it cannot be maintained in nature even under the

most ideal conditions. Therefore, artificial propagation on a large scale, once started, must be continued if proven to be practical, in order to keep the predator population on an effective control basis.

Life Cycle in Relation to Host

The average development of D. monticolae during the summer months in the sugar pine belt varies between 8 and 12 weeks from attack to exit of new adults. The larval period varies between 4 and 6 weeks. By comparison with T. virescens, with an average larval period of nearly 30 weeks, there is a high possibility that many of the predator larvae would starve unless they were able to feed on secondary insects or a successive generation of D. monticolae established further down on the bole of the same tree.

Observations made on newly infected sugar pines and freshly attacked logs in rearing cages have shown in all cases that the oviposition habits of T. virescens follow closely after the D.m. attacks. Hence the predator larvae which hatch begin to feed very shortly after D. monticolae larvae have begun development, thus taking full advantage of the short feeding period permitted by the more rapid development of the mountain pine beetle. What happens to the predator larvae after D. monticolae are gone is a question which remains unanswered. If high mortality or cannibalism is the result, the hope of a predator population increase under natural conditions, even though stimulated by artificial propagation, is minimized.

SUGGESTIONS FOR FURTHER STUDY

The experimental work on artificial propagation carried on thus far has shown definitely that through refinements of technic in the development of laboratory rearing methods, large numbers of predators can be reared to maturity. Still further development of more efficient methods of handling, provision of host food, and utilization of laboratory reared predators in the control of barkbeetle outbreaks must be developed before artificial propagation can be justified as a practical means of control.

From experience gained, the next important steps to be developed toward the perfection of this method will follow certain definite lines. Of first importance is the elimination of E. aphegus from further consideration, so that more time can be devoted to T. virescens which has proven to be very adaptable to laboratory rearing conditions.

Suggestions for improvement of methods are in order of importance: (1) development of greater efficiency in mass production of eggs through selection of consistently heavy producing females; (2) the development of an adequate host food supply at relatively low cost; and

(3) possibilities in shortening the life period of larvae. The last two suggestions will be difficult to fulfill, but nevertheless, they merit such attention. The rearing of fly larvae as a supplementary food to barkbeetles may be too expensive and possibly inadequate for development of large numbers of predators, but so far it offers more promise than any other host, and for this reason should be thoroughly investigated.

Just how much the average life period of T. virescens larvae may be shortened as the result of changes in quantity and quality of host food, temperature and other rearing conditions is a question which should be investigated completely. In experiments carried on thus far certain larvae have been seen to complete development in a short time. If the short term larvae are the result of inherent qualities this fact should be known and a method developed to rear them in quantity.

The rearing of predator larvae to mature adults may not be entirely necessary to the development and application of biological control methods. Possibly by the development of large numbers of larvae in the laboratory for liberation in the field on infested trees, control under certain conditions may be effected. So far as the hardiness and adaptability of T. virescens larvae are concerned, the possibilities entertained by this suggestion are entirely reasonable. This phase of biological control merits investigation.

SUMMARY

Studies and experiments with native predators of the mountain pine beetle in the hope of developing biological control of this species through artificial propagation were undertaken at Berkeley, California and the Miami field laboratory. The green troglitid Tamnochila virescens and the red-bellied clerid Enoclerus sphaerens were selected for this work as a result of field investigations which showed that they offered more promise than any other native insect controlling agency. Studies which were initiated in the autumn of 1937 are still in progress. Much has been learned, not only about the life history and habits of these insects, also in regard to methods and technique of handling and rearing under artificial conditions. While still far from practical, the results of the work so far on artificial propagation are very encouraging. The most important developments in this study are listed below.

1. The use of single mated pairs of each species in individual containers with a proper environment will stimulate consistent production of eggs varying in number from 6 to 60 per week per pair.

2. The most convenient and effective substratum on which eggs are laid is provided by small rolls of white crepe paper tightly wound in over-lapping layers.

3. The egg laying period as well as adult life of T. virescens

is on the average 2½ times that of E. sphageus and the number of eggs produced by T.v.c. is 3 to 4 times the number of those produced by E. sphageus.

4. The eggs of both species produced and incubated under artificial conditions are approximately 70 percent fertile.

5. The larvae of each species must be reared individually to prevent cannibalism.

6. The most satisfactory containers found are 1-dram pharmaceutical shoulder lip vials. A rearing substratum is provided by a layer of fine dry sawdust placed in the bottom of each vial.

7. With living scolytid larvae as host food the development of each species of predator is rapid and the mortality is very low.

8. Tests to determine the possibility of using other insect hosts as food, foreign to these predators, resulted in a few which were fairly satisfactory to T. virescens, but entirely unsatisfactory to E. sphageus. The most promising host yet found for a supplemental food in rearing T. virescens is larvae of the flesh fly, Lucilia sericata.

9. After feeding for 2 weeks on native host food, T. virescens will survive and develop normally to maturity on fly larvae. In this regard, E. sphageus larvae do not become adapted.

10. The average developmental period from first stage to mature larvae under laboratory conditions at a constant temperature of 75° F. is approximately 200 days for T. virescens and 46 days for E. sphageus.

11. A small percentage of each T. virescens larval group develops to maturity in approximately 50 days under the same conditions of environment and food supply as the longer lived larvae.

12. The cost in time required to rear 1,000 temnochila larvae to maturity on D. monticolae larvae as host food at Miami during 1938 was 41 man days for the long term T. virescens larvae and 29 man days for the short term larvae. A total of 23 man days per 1,000 E. sphageus larvae was required.

13. By improvements of rearing technic and use of fly larvae as supplemental food, this amount of time can be reduced by as much as 50 percent.

14. The use of E. sphageus as a possibility in artificial propagation and biological control is practically out of the question.

15. The possibilities of T. virescens are much greater, not only because of their adaptability and hardiness, but also because of their controlling ability. Their one handicap is extreme length of life cycle.



Photo #1 (9972D). Miami Laboratory, Sierra National Forest. Facilities at this field laboratory and at Berkeley were distinctly beneficial to the progress and accomplishment of experiments on artificial propagation.

- Photo by J. E. Patterson.



Photo #2 (9973J). Gas type refrigerator used in storing surplus predators and scolytids for use in artificial propagation experiments as needed.

- Photo by J. E. Patterson.



Photo #3 (9973F). Mating pairs of Tennochila virescens were easily selected for oviposition by placing a number of adults in a petri dish and holding them at room temperature. This method of selecting mating pairs has been supplanted recently by the discovery of external sexual characters.

- Photo by J. E. Patterson.



Photo #4 (9973X). Oviposition setup. One mated predator pair placed in each petri dish, which contained small fresh bark blocks of sugar pine and a few Dendroctonus adults provided an ideal stimulus for oviposition. The laborious and costly method of securing eggs has been replaced by use of crepe paper rolls instead of bark.

- Photo by J. E. Patterson.



Photo #5 (9585) Group of T. virescens eggs deposited on crepe paper. These eggs are collected simply by clipping the paper.

- Photo by J. E. Patterson.



Photo #6 (9971B). Group of T. virescens eggs deposited on bark. These were exposed by flicking away bark scales overlying them. This method of securing the eggs is laborious and in addition requires great care to prevent crushing them.

- Photo by J. E. Patterson.




Photo #7 (9973D). After the eggs have hatched, the larvae are transferred to individual vials where they are reared to maturity.

- Photo by J. E. Patterson.



Photo #8 (9973H). One-dram pharmaceutical vials each containing a predator larva. Fine, dry sawdust placed in the bottom of each vial provides an ample substratum for the predator to mill around and search for its prey. One mature host larva is provided every two days.

- Photo by J. E. Patterson.

FIGURE 1

AVERAGE SEASONAL PRODUCTION OF EGGS
FOR EACH PREDATOR

LABORATORY REARING EXPERIMENT

MIAMI - 1938

BASIS - 24 MATED PAIRS OF EACH SPECIES

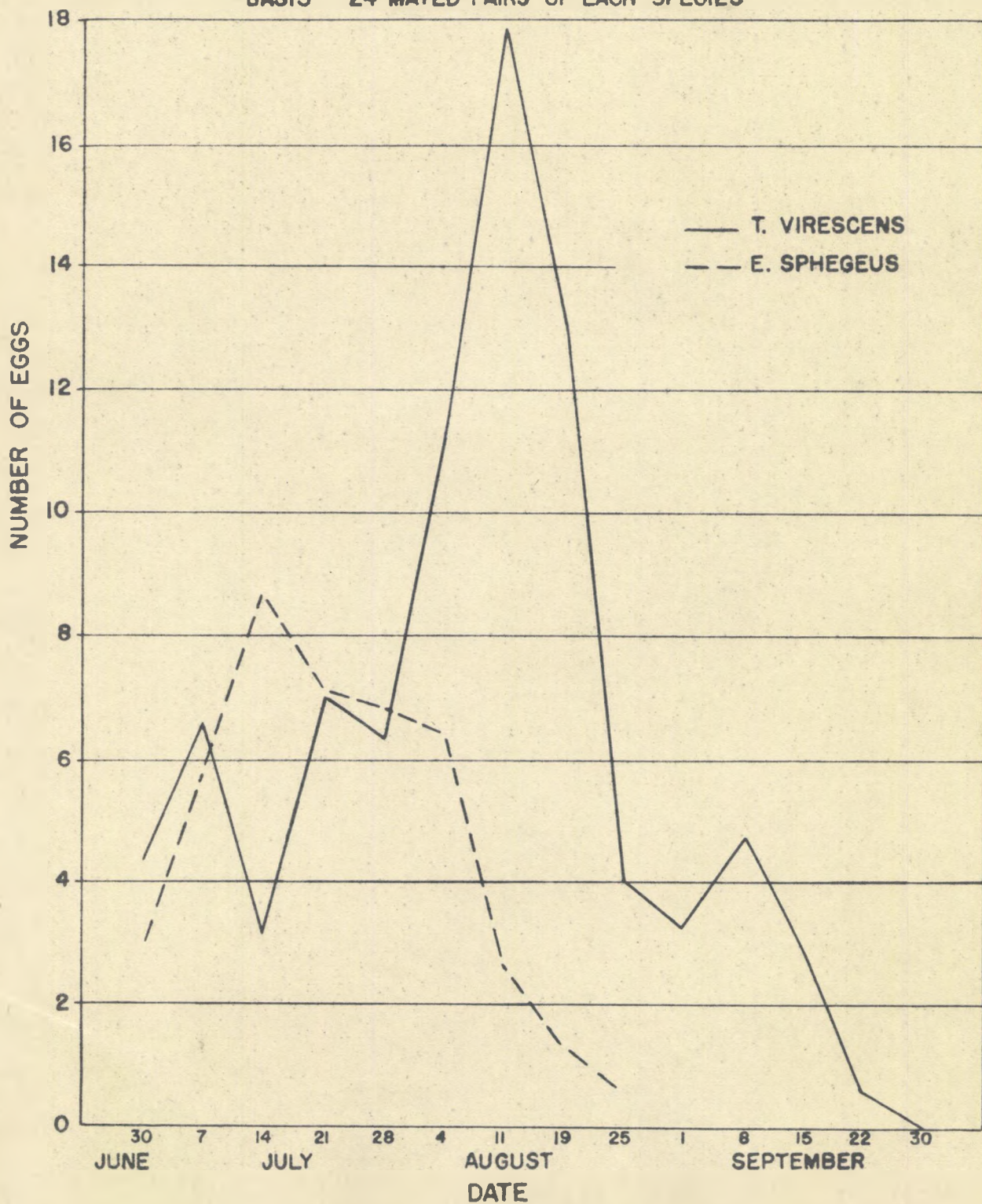


FIGURE 2

TYPICAL DEVELOPMENT OF *T. VIRESCE*

ARTIFICIALLY REARED AT A CONSTANT TEMPERATURE OF 75°F.

REARING SERIES B-E

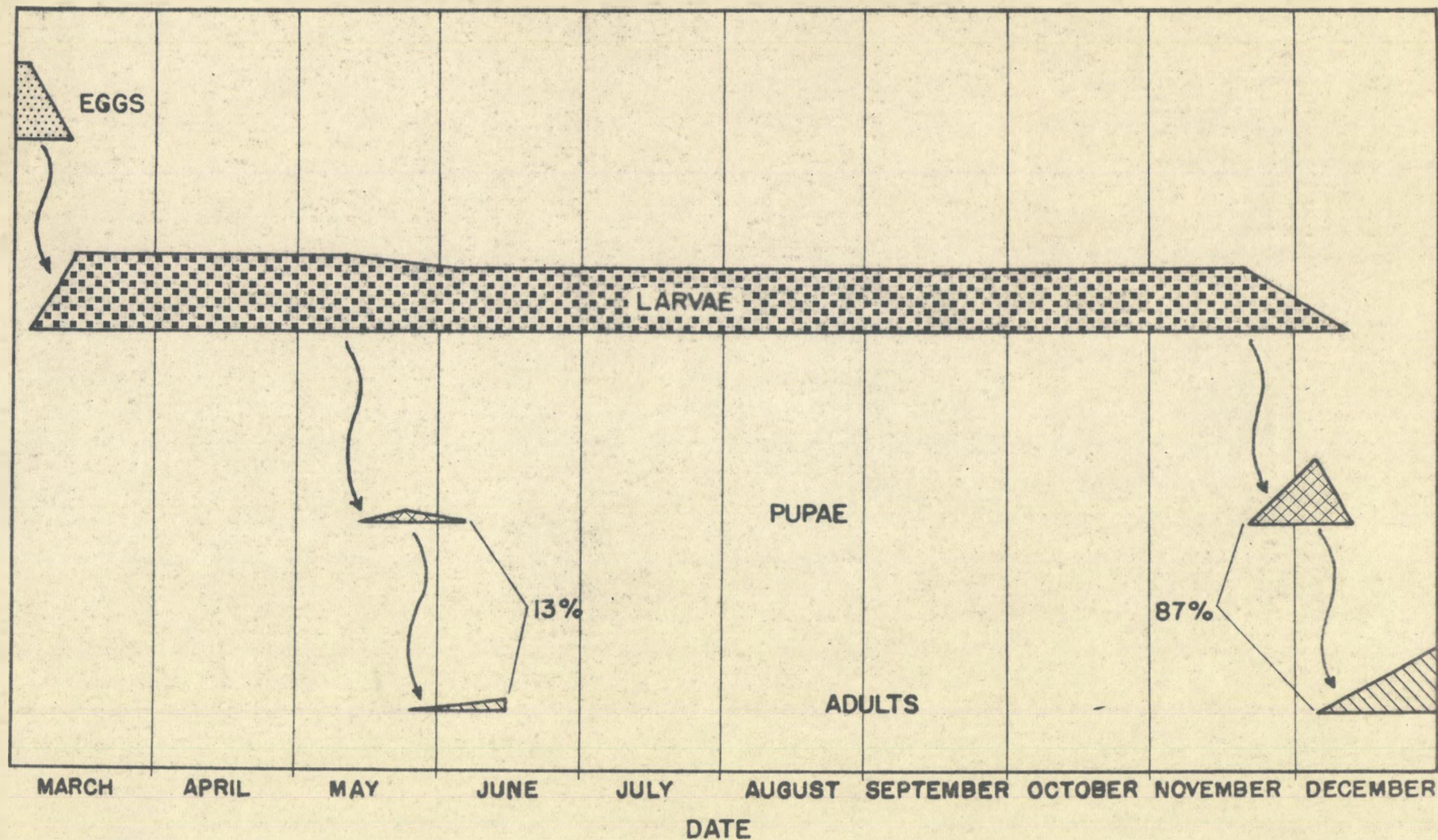


FIGURE 3

MAXIMUM AND MINIMUM TEMPERATURES

MIAMI LABORATORY — 1939

